

WHAT IS CLAIMED IS:

1        1. An isolated enone reductase having the physicochemical properties of (A)-  
2        (C):  
3            (A) it reduces the carbon-carbon double bond of an  $\alpha,\beta$ -unsaturated ketone, using  
4        NADPH as an electron donor, to produce a corresponding saturated hydrocarbon;  
5            (B) it has a substrate specificity of (1)-(4):  
6              (1) it has substantially no activity to reduce the keto group of a ketone;  
7              (2) it exhibits a significantly higher activity with NADPH than with  
8        NADH as an electron donor;  
9              (3) it does not substantially act on substrates wherein both substituents at  
10      the  $\beta$  carbon relative to the ketone are not hydrogen; and  
11      (4) it does not substantially act on a substrate in which the carbon-carbon  
12      double bond is present in a cyclic structure; and  
13      (C) it has an optimal pH of 6.5-7.0.

1        2. The enone reductase of claim 1, wherein the reductase (a) has an optimum  
2        temperature of 37-45°C; and (b) has a molecular weight determined by sodium dodecyl  
3        sulfate-polyacrylamide gel electrophoresis and by gel filtration of about 43,000 and about  
4        42,000, respectively.

1        3. The enone reductase of claim 1, which is derived from an organism of the  
2        genus *Kluyveromyces*.

1        4. A method for obtaining an enone reductase, comprising the step of  
2        (a) culturing a microorganism belonging to the genus *Kluyveromyces*; and (b) isolating the  
3        enone reductase of claim 1 from the cultured microorganism.

1        5. The method of claim 4, wherein the microorganism belonging to the genus  
2        *Kluyveromyces* is *Kluyveromyces lactis*.

1        6. An isolated nucleic acid of any one of (a) to (d) below:  
2            (a) a nucleic acid encoding a protein comprising the amino acid sequence of  
3        SEQ ID NO:2;

(b) a nucleic acid comprising a coding region of the nucleotide sequence of SEQ ID NO:1;

(c) a nucleic acid encoding a protein that comprises the amino acid sequence of SEQ ID NO: 2, in which one or more amino acids are substituted, deleted, inserted and/or added and that is functionally equivalent to a protein consisting of the amino acid sequence of SEQ ID NO: 2;

(d) a nucleic acid that hybridizes under stringent conditions with a nucleic acid consisting of the nucleotide sequence of SEQ ID NO: 1, and that encodes a protein functionally equivalent to a protein consisting of the amino acid sequence of SEQ ID NO:2; and

(e) a nucleic acid encoding a protein that has at least 60% identity to the amino acid sequence of SEQ ID NO:2.

7. An isolated nucleic acid encoding the amino acid sequence of SEQ ID NO:2

or a fragment thereof.

8 A vector comprising the nucleic acid of claim 6.

9 A vector comprising the nucleic acid of claim 7.

10. The vector of claim 8, further comprising a nucleic acid sequence encoding a dehydrogenase that catalyzes oxidation-reduction reactions using NADP as a coenzyme.

11. The vector of claim 9, further comprising a nucleic acid sequence encoding a dehydrogenase that catalyzes oxidation-reduction reactions using NADP as a coenzyme.

12. A transformant harboring the nucleic acid of claim 6.

13 A transformant harboring the nucleic acid of claim 7.

14 A transformant harboring the vector of claim 8.

14. A transitor

15. A transformant harboring the vector of claim 10.

13. A transmembrane polypeptide encoded by

16. A substantially purified polypeptide encoded by the nucleic acid of claim 1.

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1           17. A substantially purified polypeptide encoded by the nucleic acid of claim 7.

1           18. A method for producing a polypeptide, the method comprising the steps of  
2 culturing the transformant of claim 12 and recovering a polypeptide expressed from the  
3 transformant or the culture supernatant thereof.

1           19. A method for producing a polypeptide, the method comprising the steps of  
2 culturing the transformant of claim 13 and recovering a polypeptide expressed from the  
3 transformant or the culture supernatant thereof.

1           20. A method for producing a polypeptide, the method comprising the steps of  
2 culturing the transformant of claim 14 and recovering a polypeptide expressed from the  
3 transformant or the culture supernatant thereof.

1           21. A method for producing a polypeptide, the method comprising the steps of  
2 culturing the transformant of claim 15 and recovering a polypeptide expressed from the  
3 transformant or the culture supernatant thereof.

1           22. An isolated nucleic acid of any one of (a) to (d) below:

- 2           (a) a nucleic acid encoding a protein comprising the amino acid sequence of  
3 SEQ ID NO:4, 6 or 8;
- 4           (b) a nucleic acid comprising a coding region of the nucleotide sequence of  
5 SEQ ID NO:3, 5 or 7;
- 6           (c) a nucleic acid encoding a protein that comprises the amino acid sequence  
7 of SEQ ID NO:4, 6 or 8 in which one or more amino acids are substituted, deleted, inserted  
8 and/or added and that is functionally equivalent to a protein consisting of the amino acid  
9 sequence of SEQ ID NO:4, 6 or 8;
- 10          (d) a nucleic acid that hybridizes under stringent conditions with the nucleic acid  
11 consisting of the nucleotide sequence of SEQ ID NO: 3, 5 or 7, and that encodes a protein  
12 functionally equivalent to a protein consisting of the amino acid sequence of SEQ ID NO:4, 6  
13 or 8; and
- 14          (e) a nucleic acid encoding a protein that has at least 60% identity to the amino acid  
15 sequence of SEQ ID NO:4, 6 or 8.

1        23. A substantially purified polypeptide encoded by the nucleic acid of claim 22.

1        24. A vector comprising the nucleic acid of claim 22.

1        25. The vector of claim 24, further comprising a nucleic acid sequence encoding a  
2 dehydrogenase that catalyzes oxidation-reduction reactions using NADP as a coenzyme.

Sub  
A1      1        26. A transformant harboring the nucleic acid of claim 2.

1        27. A transformant harboring the vector of claim 24.

1        28. A transformant harboring the vector of claim 25.

1        29. A method for producing a polypeptide, the method comprising the steps of  
2 culturing the transformant of claim 26 and recovering a polypeptide expressed from the  
3 transformant or the culture supernatant thereof.

1        30. A method for producing a polypeptide, the method comprising the steps of  
2 culturing the transformant of claim 27 and recovering a polypeptide expressed from the  
3 transformant or the culture supernatant thereof.

1        31. A method for selectively reducing the carbon-carbon double bond of an  
2  $\alpha,\beta$ -unsaturated ketone, comprising the step of reacting an  $\alpha,\beta$ -unsaturated ketone with the  
3 enone reductase of claim 1.

1        32. A method for selectively reducing the carbon-carbon double bond of an  
2  $\alpha,\beta$ -unsaturated ketone, comprising the step of reacting an  $\alpha,\beta$ -unsaturated ketone with the  
3 polypeptide of claim 16.

1        33. A method for selectively reducing the carbon-carbon double bond of an  
2  $\alpha,\beta$ -unsaturated ketone, comprising the step of reacting an  $\alpha,\beta$ -unsaturated ketone with the  
3 polypeptide of claim 17.

1        34. A method for selectively reducing the carbon-carbon double bond of an  
2  $\alpha,\beta$ -unsaturated ketone, comprising the step of reacting an  $\alpha,\beta$ -unsaturated ketone with the  
3 polypeptide of claim 23.



(2) it exhibits a significantly higher activity with NADPH than with

NADH as an electron donor; illustrates wherein both substituents at

(3) it does not substantially act on substrates wherein both substituents at the 1, 2-positions are hydrogen; and

the  $\beta$  carbon relative to the ketone are not hydrogen; and

(4) it does not substantially act on a substrate in which the carbon-carbon bond is present in a cyclic structure; and

(C) it has an optimal pH of 6.5-7.0.

(C) 40. The method of claim 38, wherein the microorganism is of the genus

### *Kluyveromyces.*

41. The method of claim 38, wherein the microorganism is the transformant of

claim 12.

The method of claim 38, wherein the microorganism is the transformant of

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claim 12.

42. The method of claim 38, wherein the microorganism is the transformant of  
claim 26.